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## EXPERIMENTAL EPIDEMIOLOGY IN TUBERCULOSIS \*

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The keen interest shown in the study of epidemiology since medicine realized the idea of the infectivity of some diseases, demonstrates the importance of those studies from theoretic as well as from practical points of view. Particularly in tuberculosis that study has been carried on with much intensity. The enormous quantity of literature accumulated on the subject, its exhaustive treatment in so many excellent books, leads to a postulate, namely: The longer a community of people has been in contact with tuberculosis virus the less mortality is to be found among the persons constituting that community, tho almost 90% of civilized people in postmortem examination present healed tubercular lesions.

If we ask the cause of this diminishing mortality, we may inquire (1) whether the virus has become attenuated through passages, or (2) whether the persons concerned have acquired a resistance to the virus. These questions have been left without answer, and quite naturally, because observations or experiments on human beings are based on insufficient determinism, the subjects are never comparable, and we are unable to ascertain how far diseases are antagonistic to each other. It would be of great interest to put these problems on an experimental footing, and thereby ascertain the biologic laws which govern those established postulates; in other words, we could inquire how tuberculous process is spread, what are the probable channels of infection, and what are the conditions under which resistance or immunity is established. Those were the problems in my mind, and I give in this work the first results of my investigations.

The experiments carried out were extremely simple. Guinea-pigs were injected with a known dose of tubercle bacilli, and then fresh guinea-pigs coming straight from the caviary were put into the same cages at fixed intervals. No special precautions were taken as regards cleanliness. Small and large cages were cleaned every 7 days. The large cages were 60 cu. in. square, and the small cages measured 30 cu. in.

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## EXPERIMENTS

- 1.—A. 11/27/1914. Four guinea-pigs were injected subcutaneously with 0.1 mg. of tubercle bacilli each.
- 12/14. One guinea-pig found dead with generalized tuberculosis.
- B. 12/14. Four guinea-pigs arriving straight from the caviary were put in the same cage.
- A. 12/20. One found dead with tuberculous lesions in the liver and the spleen.
- B. 12/20. Two dead. Lesions in the spleen and the liver.
- C. Emulsion of the spleens of these two guinea-pigs injected into 2 fresh guinea-pigs.
- B. 12/21. Another guinea-pig dead with generalized tuberculosis.
- D. Emulsion of the spleen of the animal in B injected into 2 fresh guinea-pigs.
- C. 12/29. Found dead with very pronounced lesions.
- D. 1/ 4/1915. Killed. Generalized tuberculosis.
- B. 1/29. The last guinea-pig of B killed. Only small lesions in the spleen. Weight of animal normal.

It was decided to kill B4 because of its comparatively long life. At any rate, some doubt may exist as to whether the disease was retrogressing, beginning, or stopping; but the normal weight of this animal and its long life indicate that probably there was a slight tuberculous process from the beginning which had been mastered by the animal. Some of the guinea-pigs of Experiment A were living on Feb. 15, 1915. They were then employed for other experiments.

The injection of the emulsion of the spleen of the dead animals as mentioned gave positive results. In the following experiments only sections were made to confirm the microscopic lesions. From this experiment it is seen that of the fresh guinea-pigs put in contact with tubercular guinea-pigs, after 17 days of infection, 75% die with the utmost rapidity and exhibit tuberculous lesions on postmortem examination.

- 2.—A. 11/27/1914. Four guinea-pigs injected subcutaneously with 0.05 mg. of tubercle bacilli (half the dose used in Experiment 1).
- A. 12/ 5. One guinea-pig found dead. Small lesions in the spleen.
- B. 12/14. Four fresh guinea-pigs put into this cage.
- B. 12/15. One found dead. No lesions.
- B. 12/17. Another guinea-pig dead. Lesions in the spleen, and in the lung.
- B. 12/21. Another guinea-pig dead. Lesions in the spleen and in the liver.
- B. 1/12/1915. The last dead. Generalized tuberculosis and mesenteric ganglion. The spleen not enlarged.
- A. Three still living on March 10, 1915, when they were employed for other experiments.

This experiment confirms the first, and shows that the amount of the initial dose of tubercle bacilli does not materially affect infection by contact, and that infection by contact in 100% of the cases is lethal. Contacts die in less time than injected guinea-pigs.

- 3.—A. 11/27/1914. Four guinea-pigs injected subcutaneously with 0.1 mg. of tubercle bacilli.  
 B. 12/30. Two fresh guinea-pigs put in as contacts.  
 B. 1/25/1915. One found dead. Generalized tuberculosis and mesenteric ganglion.  
 B. 2/1. The other killed. Lesions in liver, lungs, and spleen. Spleen not enlarged. Large mesenteric ganglion without pus.

This experiment confirms the first two. The conditions of infection are still active after 1 month and 3 days.

- 4.—A. 12/17/1914. Six guinea-pigs injected with 1 mg. of tubercle bacilli.  
 B. 12/30. Two fresh guinea-pigs put into the same cage.  
 B. 1/28/1915. One dead. Generalized lesions. Mesenteric ganglion.  
 B. 2/1. The other killed. Only mesenteric ganglion.

It seems from this experiment that the virus is infectious 13 days after the infection, at least in 50% of the cases.

In B 2 as in B 4 of the first experiment, the weight was normal, and the lesions small. The guinea-pigs left in all these experiments were killed because it was necessary to determine first of all whether every contact was infected.

The questions arising in connection with these slightly infected animals are a subject for further inquiries.

- 5.—A. 3/27/1915. Four guinea-pigs injected subcutaneously with 1 mg. of tubercle bacilli.  
 4/28. One guinea-pig dead with generalized tuberculosis.  
 B. 4/28. Two newly arrived guinea-pigs put into the cage.  
 A. 5/3. Another guinea-pig found dead with lesion in the spleen.  
 A. 5/10. Another dead, with generalized tuberculosis.  
 6/8. The guinea-pigs left were killed. A—generalized tuberculosis; B—only small lesions in the lungs.

These experiments demonstrate that the virus is infectious from the 13th to the 33rd day. It was next desirable to inquire at what time the virus commences to be infective for contacts, and also when the virus becomes no longer infective.

- 6.—A. 5/27/1915. Eight guinea-pigs injected subcutaneously with 1 mg. of tubercle bacilli.  
 B. 6/17. Two contacts.  
 7/8. Killed because they were losing weight. Lesions in the lungs of one very evident. One part of the lungs of the other and the spleen affected.

This also confirms the previous experiment; the virus is still infective after 21 days from the infection.

- 7.—A. 4/22/1915. Six guinea-pigs injected with 0.5 mg. of tubercle bacilli.  
 B. 5/18. Three fresh guinea-pigs for contact.  
 A. 6/25. Dead.  
 B. 7/8. Killed. No lesions.

This experiment shows how difficult it is in biology to group observations in accordance with a rigid law. A certain amount of amplitude must be allowed for the experiments on account of virulence of the strains of tubercle bacilli and on account of individual factors, etc.

- 8.—A. 5/21/1915. Four guinea-pigs injected with 0.5 mg. of tubercle bacilli.  
 B. 6/9. Two fresh guinea-pigs for contact.  
 A. 7/2. Dead.  
 B. 7/8. Killed. No lesions.

This experiment follows No. 7, but in the majority of the experiments it can be said that an infection arises through contact. We therefore proceeded to inquire when the virus commences to be infectious, and when it ceases to be so.

- 9.—A. 7/17/1915. Four guinea-pigs injected with 1 mg. of tubercle bacilli.  
 B. 7/23.  
 C. 7/26.  
 D. 7/29.  
 E. 8/10.  
 F. 8/20. } Two healthy guinea-pigs arrived from the caviary as contacts.  
 A. 8/23. All dead.  
 E. 8/18. Both dead with pulmonary tuberculosis.  
 D. 8/24. One dead. No lesions except cachexia.  
 8/24. All the injected animals being dead, I resolved to kill the contacts.

The results were as follows: B—no lesions; C—(1) lesions in the spleen, (2) no lesions; D—the guinea-pig left had lesions in the lungs; F—no macroscopic lesions.

From these experiments it seems that the virus is absolutely infectious from the 9th to the 24th day after injection.

- 10.—A. 7/17/1915. Four guinea-pigs injected with 1 mg. of tubercle bacilli.  
 B. 7/20.  
 C. 7/23.  
 D. 8/10.  
 E. 8/20. } Two healthy animals introduced into this cage.  
 A. 8/30. All dead.  
 B. }  
 C. } 8/31. Killed.  
 D. }  
 E. }  
 B. 8/31. Negative.  
 C. 8/31. Negative.  
 D. 8/31. One showed lesions in the spleen, the other had lungs affected.  
 E. 8/31. One had lungs affected, the other revealed no lesions.

This experiment seems to confirm No. 9. There is a cycle in the infectivity of the tuberculous virus which is nil at the beginning, and which then increases its infectivity with the multiplication of the tubercle bacilli in the body of the infected animal. Now the question arises whether the virus is infective at the beginning and at the end of the process.

- 11.—A. 6/ 9/1915. Six guinea-pigs injected each with 1 mg. of tubercle bacilli.  
B. 6/ 9. Two healthy animals introduced into this cage.  
A. 7/ 8. One dead with generalized tuberculosis.  
B. 7/ 8. Killed. No lesions.  
C. 7/14. Two fresh contacts.  
C. 8/31. Killed. No lesions.

This experiment shows (1) that fresh guinea-pigs put in contact with tuberculous guinea-pigs on the first day of the injection do not become tuberculous, and (2) that after 1 month and 22 days, the virus ceases to be infectious.

- 12.—A. 8/21/1915. Six guinea-pigs injected with 1 mg. of tubercle bacilli.  
B. 8/21. Three guinea-pigs for contact were put into the same cage.  
B. 8/ 3. One of the contacts was lost.  
A. 10/ 4. All dead with tuberculous lesions.  
B. 10/ 4. One found dead. No tubercular lesions; lungs full of gas.  
B. 10/ 4. The contact left was killed. No lesions.

These experiments confirm No. 11.

- 13.—A. 1/29/1915. Three guinea-pigs injected with 1 mg. of tubercle bacilli.  
B. 1/29. Three guinea-pigs for contact.  
A. 3/18. Dead with generalized tuberculosis.  
B. 4/20. No signs of diminution of weight, therefore animals tried for immunity (see No. 16).  
14.—A. 1/29/1915. Three guinea-pigs injected subcutaneously with 1 mg. of tubercle bacilli each.  
B. 1/29. Three fresh guinea-pigs for contact.  
A. 3/18. One dead with generalized tuberculosis.  
B. 4/20. No signs of diminution of weight. Tried for immunity (see No. 16).  
15.—A. 1/20/1915. Four guinea-pigs injected with 1 mg. of tubercle bacilli each subcutaneously.  
B. 1/20. Two guinea-pigs for contact.  
B. 2/ 9. One found dead. No apparent tuberculous lesions. The small intestine as in diarrhea.  
B. 4/20. The other guinea-pig showed no diminution of weight, and therefore was used for trial of immunity (see No. 16).

The following experiment was undertaken to ascertain whether guinea-pigs living for a long time in contact with tuberculous guinea-pigs acquire a state of immunity.

- 16.— { A1 from Experiment 15, injected with 0.1 mg. of living tubercle bacilli.  
 4/20/1915. { A2 from Experiment 14, not injected.  
 { A3 from Experiment 14, injected as before.  
 { A4 from Experiment 14, injected as before.  
 { A5 from Experiment 13, not injected.  
 { A6 from Experiment 13, injected as before.  
 { A7 from Experiment 13, injected as before.  
 { B. Two guinea-pig controls injected each with 0.1 mg. of living tubercle bacilli.  
 5/ 4. A1 found dead, cachectic. No macroscopic lesions were observed.  
 5/15. B1 found dead. One spot in the lungs; very cachectic.  
 5/18. A3 found dead. Much emaciated; a small ganglion under the sternum, without pus.  
 6/18. A4 found dead. Generalized tuberculosis. Pancreas also affected.  
 7/ 2. The other four and the controls killed. A2—no trace of lesions; A5—no trace of lesions; A7—generalized tuberculosis; and B2—generalized tuberculosis.

This experiment confirms the last one on the one hand and shows that for a demonstration of immunity subcutaneous inoculation is too severe a test. We hope to return to this matter later on.

The following experiments were made to ascertain whether after a considerable interval of time, the virus of tuberculosis is still infectious.

- 17.—A. 2/ 5/1915. Four guinea-pigs injected with 0.1 mg. of tubercle bacilli.  
 B. 4/20. A fresh guinea-pig is put in.  
 B. 6/ 9. Killed. No lesions.

The result of this experiment indicates that the older the disease, the less dangerous it is for the contacts. In fact, after 1 month and 15 days, the virus is no longer infectious.

- 18.—A. 5/27/1915. Eight guinea-pigs injected with 1 mg. of tubercle bacilli.  
 B. 7/14. Two fresh guinea-pigs for contact just arrived.  
 B. 8/31. Killed. No lesions at all in either.  
 19.—A. 5/21/1915. Four guinea-pigs injected with tubercle bacilli.  
 B. 7/14. Two contacts arrived from the caviary.  
 B. 8/31. Killed. No lesions in either.

It seems from these last experiments that a cycle of the infection through contact exists. There is a curve which is nil at the beginning, which rises to its highest point between the 9th and the 33rd days, and which then goes down again. As we have pointed out before, these experiments indicate that there is a biologic law, with wide variations due to the influence of environment, etc., which governs the results.

The next set of experiments was undertaken to ascertain the relationship between mother guinea-pigs and their young. It is regretted

that the following experiments are few in number, because the breeding went wrong.

- 20.—3/12/1915. A mother guinea-pig was injected with 1 mg. of tubercle bacilli. The 3 young, which were 4 days old, were left in her cage.
- 3/15. One of the young dead. No lesions. Von Pirquet test on the two left.
  - 4/19. Both dead. No lesions. The reaction was negative. The mother living, and used for other experiments.

It has frequently occurred to me that the young die for very little cause. In this case the shock of the carrying out of the von Pirquet test probably caused the death of the young guinea-pigs.

- 21.—3/12/15. A mother guinea-pig injected with 1 mg. of tubercle bacilli. Three young, which were 4 days old when the mother was injected, were left in her cage.
- 4/12. Mother dead. Lesions in lungs. Very cachectic.
  - 4/27. One young dead with no lesions.
  - 4/27. The two left put into a big cage with 6 guinea-pigs which had been injected with tubercle bacilli 1 month before.
  - 4/30. One of the young dead without lesions. Possibly trampled to death.
  - 6/15. The injected guinea-pigs died. The young guinea-pig put into another cage along with guinea-pigs injected 20 days before.
  - 8/28. The young one left was killed. No lesions.

This last result is exceedingly interesting, if it is confirmed by subsequent experiments.

- 22.—1/20/1915. A mother guinea-pig injected subcutaneously with 1 mg. of tubercle bacilli. Her two young, 2 days old, put with her.
- 2/9. Another fresh full-grown guinea-pig put into the same cage (small cage normally containing 2 guinea-pigs).
  - 2/19. One of the young dead. No lesions.
  - 2/24. Mother has ganglion and tumor at the point of inoculation.
  - 3/26. The other young one dead. No lesions.
  - 4/19. The third contact found dead. No lesions, but very emaciated. Mother used for other experiments.

The results of these experiments summed up indicate that the young had not suffered from tuberculosis at all, that they probably had attained immunity, tho I realize that I have not made enough experiments to warrant a conclusion.

#### CHANNEL OF INFECTION

A point which deserves investigation in connection with these experiments is how contact guinea-pigs become infected. Three hypotheses are possible: infection occurs (1) through the mouth, (2) through the nose, or (3) through both.



The following observations may enable us to discriminate between the three hypotheses: (1) Guinea-pigs never eat the hay which they have trampled on even when they are very hungry. (2) They never eat their own feces. (3) They never clean themselves as many other animals do. (4) When they are lively, they dig up the hay with closed mouth. (5) They never cough. (6) But when they are idle, they very often put themselves mouth against mouth.

The last observation suggested that the expired air contained the infective virus, and this is very probably the way in which they infect each other. It would mean that the channel of infection was chiefly the nose. The following experiments were designed to demonstrate this point:

- 23.—1/28/1915. One five-hundredths milligram of a 3-months-old culture put into the nose of each of 2 guinea-pigs.
- 3/ 1. One dead. Lesions in the spleen, which is of normal size, in the liver and lungs. Mesenteric ganglia.
  - 3/ 8. The other killed. No macroscopic lesions.
- 24.—3/12/1915. Six guinea-pigs were instilled by way of the nose with 0.05 mg. of tubercle bacilli in one dose each. Divided into 3 lots of 2 each, A, B, and C.
- 3/16. Lot A left as control. Lots B and C were instilled again with the same quantity.
  - 3/20. One of Lot B dead with pulmonary lesions only.
  - 3/30. The two of Lot C instilled again with the same quantity.
  - 4/28. One of Lot C dead. No macroscopic lesions.
  - 8/ 6. Animals killed. A1—very small lesions in the lungs; A2—very many lesions in the lungs; B—lungs and spleen affected; and C—rare lesions in the lungs.

#### FEEDING EXPERIMENTS

- 25.— The same as before, except that the doses were doubled.
- 8/6/1915. Animals killed. No lesions.

#### CONCLUSIONS

Guinea-pigs can become infected through contact.

The infectivity follows a curve which is nil at the beginning of the infection, and goes on to the acme, and afterwards is no longer dangerous.

It seems that at the beginning of the process few tubercle bacilli are excreted, with which the new contact can easily deal, and acquire a kind of resistance which preserves the animal when the excretion is at its acme; but as soon as the infection goes on and the microbes swarm in the body, then massive doses are excreted. By this time the

new contact is powerless to cope at once with this large quantity, and therefore the pathogenic process establishes itself, and this is made worse by the daily absorption of great quantities of virus.

In these experiments, this condition arose between the 9th and the 33rd days after the infection.

After this period a puzzling condition was observed. The guinea-pigs no longer contracted the disease. It was assumed that at about this time the process of the encapsulation of the lesions began. Up to this time the virus had been wandering in the organism; after that period the organism had the power to form around the virus a wall which only let through the toxin, and perhaps a few microbes, or none, were excreted. Then the contacts could no longer be infected.

We have tried to give an experimental basis for the fact that the channel of infection in tuberculosis in guinea-pigs is chiefly the nose. In fact, extremely small doses which, given through the mouth, have no effect on the animals, given through the nose, almost certainly produce pulmonary tuberculosis.

We do not disregard the possible hypothesis that both channels of infection may help each other to bring the process more quickly to an end. It is known that small doses of tubercle bacilli repeatedly given through the mouth are more effective than a big dose given at one time. On the other hand, in our experiments small doses per os did not produce any trace of lesions, whereas the half of these given through the nose made guinea-pigs definitely tuberculous (Nos. 23, 24, and 25). Moreover, in these experiments the guinea-pigs, whether infected through the nose or by contact, presented pulmonary lesions. If we apply these results to our knowledge acquired in epidemiology in human beings, we must acknowledge that the second phase in our experiments is amply demonstrated. If we send tuberculous people to a country where there is no tuberculosis, the people of the country in question are affected in the same way as our guinea-pigs when they were put in contact with guinea-pigs already infected from the 9th to the 33rd day, and subsequently they die from an acute form of the disease and in a very short time.

As regards the first phase we have no observations to be compared with conditions obtaining among civilized people. This is an experimental condition which it would be difficult to obtain in observations of human beings. However, most probably we have similar conditions in people who excrete few tubercle bacilli. They infect their

neighborhood, but because of the small quantity excreted the contact organism can deal with it, and probably there arises that condition of immunity of which we hear frequently in human beings.

As regards the third phase, that guinea-pigs do not get infected in the last period, it is again difficult to find the corresponding phase in human beings, but we believe that there is probably a similar condition shown by cachectic tuberculous patients. At any rate, this is a problem which deserves inquiry, as also does that of the immunity arising in contacts.